Art. XV.—*The Estimation of Acidity.*

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(Communicated by Professor W. A. Osborne, M.B., B.Ch., D.Sc.)

[Read 9th December, 1920.]

Three methods are in practice available for the determination of the acidity or basicity of solutions, i.e., their hydrogen ion concentration :—

> (1) The use of a " ladder " of indicators.
> (2) The hydrolysis of esters.
> (3) The electrometric method.

(1) The employment of a "ladder" of " Indicators" which give definite color changes at different hydrogen ion concentration.

These properties, which are possessed by many of the aniline dyes, have been employed by Salm. and Sörensen in arranging a scheme which makes use of about fourteen of these substances, the authors mentioned having placed them in a table, showing the indicators available, and the concentrations which their colour changes cover. This table, which has been altered and improved by Bayliss, will be found in his " Principles of General Physiology," (1), and the student is recommended to examine it. On inspection it will be seen that as many as four changes of hydrogen ion concentration may be made apparent with one indicator, and these are so arranged that there is a definite gradation in the series, from high, i.e. acid, concentrations, through neutrality, to the low concentrations of alkaline solutions, which, in spite of the seeming paradox, possess a demonstrable hydrogen ion concentration.

By means of these indicators, concentrations ranging from $N \times 2$ to $N \times 10^{-12}$, may be measured and some idea of the principles involved in the arrangement of the " ladder " may be gained when it is pointed out that, whereas crystal violet in solution becomes golden yellow at a hydrogen ion concentration of

at $Nx10^{-2}$, and violet at $Nx10^{-3}$; undergoing no further change $Nx2$, it becomes green at one of $Nx1$, green blue at $Nx10^{-1}$, blue in decreasing concentrations. Again, phenol-phthalein is colourless in hydrogen ion concentrations, ranging from $Nx2$ to $Nx10^{-8}$, but it becomes rose coloured at $Nx10^{-9}$, red at $Nx10^{-10}$, and thereafter remains unaltered.

	PH10	Reactions of fluids	Optimum reactions
	9.5		
	9		
	8.5	Intestinal contents	
		Pancreatic juice	
	8		Trypsin on fibrin
			Erepsin
	7.5	Blood	
	7	Human milk	Neutrality
		Saliva	Maltase
		Cow's milk	Ptyalin
	6.5		Trypsin /casein
	6	Urine	
	5.5		
	5	Infants gastric juice	
	4.5		Invertase
	4	0.0001N.HCl (4.01)	
		0.001N.acetic (3.87)	
	3.5		
	3	0.001N.HCl.(3.01)	
		0.1N.acetic (2.87)	
	2.5	N.acetic acid (2.37)	
	2	0.01N.HCl (2.02)	
	1		
	1.5	Adult	Pepsin
		gastric juice(0.9-1.08)	

Indicators (left column, top to bottom): PHENOLPHTHALEIN, CRESOL RED, PHENOL RED, Br.Thymol Blue, Br.Cresol Purple, METHYL RED, BROM-PHENOL BLUE, THYMOL BLUE

It will thus be seen that by the selection of a suitable indicator sensitive over changes of hydrogen ion concentration which may be anticipated, accurate information as to the ionic concentration of an unknown solution may be gained.

A " ladder " of indicators arranged by the writer, somewhat on the lines of that in S. W. Cole's Practical Physiological Chemistry, 5th edition, is illustrated in figure 1. The dyes there mentioned are prepared by the Cooper Laboratory, Watford, who reproduce the scheme referred to.

If this " ladder " be examined, it will be seen that the indicators there shown cover a wide range of possible hydrogen ion concentration, and it may be of interest to note certain of the features of some of the indicators in this particular series.

Methyl red and thymol blue, for instance, may be used in the precipitation of an amphoteric substance, such as anthranilic acid from its alkaline solution. In this operation, insufficient mineral acid will not completely precipitate the organic acid, while an excess will redissolve it. By the use of these indicators the exact point is readily found. To do this one employs a test paper saturated with methyl red, making spot tests upon it from time to time, until the acid point is approached; then the addition of acid is further continued until a thymol blue paper also shows an acid reaction, which is the concentration of acid at which the maximum precipitation takes place. In thymol blue we have an indicator of two useful working ranges, i.e., an acid and an alkaline range, which can be employed in differential acidimetric and alkalimetric titrations. Any acid with a dissociation constant equal to or less than acetic acid, can be titrated in the presence of hydrochloric acid if the weaker acid is completely neutralised when the alkaline change of indicator occurs. (2)

One illustration taken from a communication by Clark and Lubs will serve to show the application of this method to the differential titration of an organic acid in the presence of hydrochloric acid.

Many organic acids are not dissociated at a concentration of hydrogen ions at which hydrochloric acid is itself completely dissociated. If to a saturated aqueous solution of benzoic acid a known amount of indicator be added, and this is used as an indicator colour standard, the hydrochloric acid content of an unknown mixture may be estimated by titration with normal sodium hydroxide, in the presence of the same indicator, until the colours are matched. Care must be taken that the indicator

tion of hydrogen ion concentration. Its sensitiveness is remarkable, and this will perhaps be better realised when it is pointed out that its use has made possible as an ordinary laboratory procedure the estimation of the change in the hydrogen ion concentration in the blood, which controls the mechanism of respiration. (5)

The method is free from the objections inseparable from the use of colorimetric methods, and it may be used to determine with great exactitude the end point in a titration, where a definite cencentration of hydrogen ions is aimed at. Furthermore, since no reagents are introduced, the solution undergoing investigation remains the same at the end of the examination as at the beginning.

In comparing the electrometric with the colorimetric method this very important fact must be kept in mind—titration methods exhibit the total amount of acid or alkali present, i.e. dissociated, plus undissociated, since with every addition of standard acid or alkali chemical combination takes place, and a further portion of the dissolved substance becomes dissociated. This process will continue until the whole of the reacting acid or base is neutralised, and thus, not the actual, but the available amount of reacting salt is shown.

The routine use of such an exact method has become a necessity to the biochemical worker. To take a case in point, one of the notable advances in both human and comparative physiology has been the recognition of the great importance which attaches to the maintenance of the blood at an hydrogen ion concentration, which varies only within the narrowest limits. In the blood, an elaborate mechanism, involving the " buffer " action of its contained carbonates and phosphates exists for this purpose. As Bayliss points out, in the case of the frog, " the heart is affected by so small a change in hydrogen ion concentration as that from neutrality, $Hx10^{-7.7}$, to one of $Hx10^{-6.5}$, while a rise in H. concentration corresponding to that effected by adding 0.036 mgm. of HCl to a litre of distilled water, i.e., to $Hx10^{-6}$, is fatal."

To quote Bayliss's words, " If we were dealing with distilled water only, the addition of one-millionth of a gram molecule of HCl to a litre of distilled water would raise its hydrogen ion concentration from $Hx10^{-7}$ to $Hx10^{-6.5}$, and such a change as this represents would be ten times in excess of that which would be fatal to many protoplasmic processes."(1)

The electrometric method is of service in investigations involving the optimum reaction of enzymes.

Recently its application to the preparation of bacteriological
media having a prearranged exact hydrogen ion concentration
has proved to be of the greatest utility. Beside this, the investiga-
tion of the acidity of soil extracts, which is now known to bear
an important relation to plant growth, has been shown to be
another field in which the method should be of great use.(6)

In the presence of these facts it will be evident that an instru-
ment of precision for the measurement of such slight variations
in hydrogen ion concentration is of the greatest value to the bio-
logical chemist, and it is safe to assume that the hydrogen elec-
trode will also occupy an important place in the clinical armamen-
tarium of the future.

Theory of Electrode Potential.

Before proceeding to a description of the hydrogen electrode
in detail, it will be necessary to discuss in general the prin-
ciples which underlie its use. The practical application of the
method received a great impulse from the work of Nernst, who
showed that when a metal made contact with a solution of one of
its salts, the electric charge, or potential, which it acquires with
respect to the solution, bears a constant relation to the concen-
tration of the dissolved salt.

As this law holds good whatever the salt may be, it is evident
that the e.m.f. developed depends only upon the concentration
in the solution of ions identical with the metal employed.(7)

When any substance goes into solution—and all substances ex-
hibit a certain tendency to dissolve—we must conceive the process
to be accompanied by electrical phenomena.

In the case of a soluble salt, its solution will be followed in-
stantly by the dissociation of a certain fraction into ions bearing
complementary charges of electricity. As such a system is in a
state of equilibrium, the electrical phenomena are not manifest.

Where metals are concerned, however, the case is different,
since a metal, when dissolving, can only give off to the solution
positively charged ions, and, depending on the degree to which
it does so, the metal will become negatively charged.

We may take a concrete case. Let us suppose a rod of zinc
to make contact with a solution of the chloride of this metal.
Two opposing forces now come into operation. One is repre-
sented by a tendency of the zinc rod to go into solution, and
become to this extent negatively charged, i.e., to give off posi-

tively charged ions to the solution. The other is a tendency for the dissociated zinc to abandon the ionic form, and to appear upon the rod in the metallic state; but while this latter will be recognised as the osmotic pressure of the dissolved metal, the former has been termed by Nernst the " electrolytic solution pressure " of the metal in question.

It will be evident then, that where the osmotic pressure of the dissolved salt exceeds the electrolytic solution pressure of the metal, the plus charges imparted in this way to the rod will cause it to become positively charged with respect to the solution.

It must be understood that owing to electrostatic attraction, the plus charged ions cannot move more than an infinitesimally small distance from the rod unless an external circuit be provided, when a current will pass until equilibrium is established. Until the external circuit is closed we must assume the existence of a " Helmholtz double layer."

We may perhaps make the matter clearer and trace a closer correspondence between theory and practice by studying the development of an e.m.f. in such a cell as the Daniell.

The Daniell cell consists of a glass vessel, within which is placed a porous cylinder.

The outer vessel contains a solution of copper sulphate in which is immersed a copper plate, while the inner vessel is supplied with a solution of zinc sulphate in which rests an electrode of zinc.

If electrical connection be made between the zinc and the copper plate, a current will follow, and a galvanometer inserted in the circuit will indicate an e.m.f. of 1.1 volt.

The production of this e.m.f. may be explained thus:—

Copper exhibits in solution a strong tendency to abandon the ionic form, and its electrolytic solution pressure is therefore low, being lower than its osmotic pressure. In consequence of this a copper electrode is positive to solutions of its salts.

On the other hand, as we have seen, the zinc readily gives off positively charged ions to its solution, and its charge is therefore negative.

The combination in the Daniell cell, i.e., copper—copper sulphate; zinc—zinc sulphate, may be represented graphically thus:
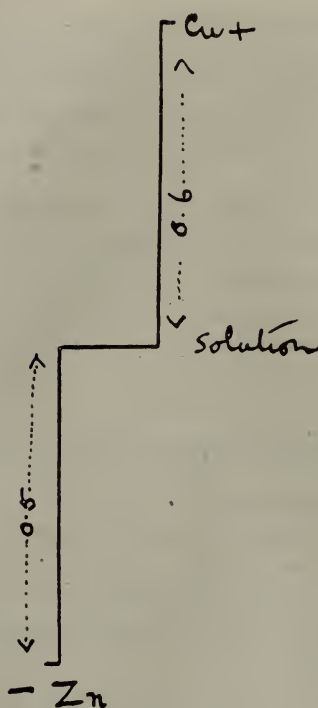
Fig. 2.   (After Lehfeldt)

The sum of the differences thus shown is 1.1 volt, which is the known voltage of the Daniell cell.

Concentration Battery

Knowing the laws which govern the production of e.m.f. it is possible to set up an arrangement known as a " concentration cell," in which both electrodes consist of the same metal. These are made to dip into vessels containing a solution of a salt of the metal at differing concentrations. Electrical connection is made between the vessels by suitable means. The arrangement shown below may be employed, where a suitably bent glass tube contains the solutions, means being employed at *c* to prevent rapid diffusion. Let the electrodes at *a,* and *b,* for example, consist of silver, and the solutions be of some salt of that metal.

If, now, the electrodes be connected with a galvanometer, an e.m.f. will become apparent, which, as far as the external circuit is concerned, is directed toward that electrode which dips into the more dilute solution.
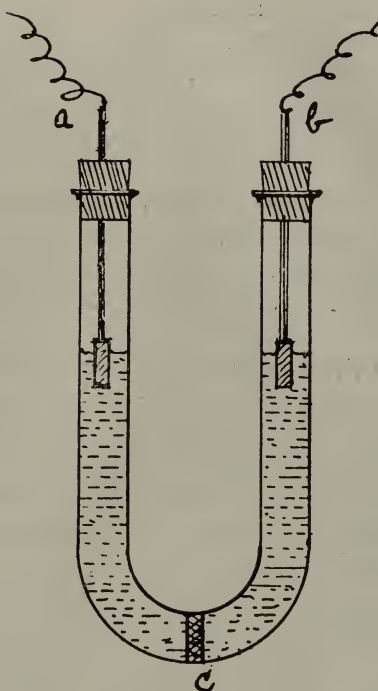
Fig. 3.

The production of an e.m.f. in this case, and its direction, depend upon the fact that while both electrodes give off positively charged ions to the solution, and to this extent become negatively charged themselves, the electrode, which is in contact with the more dilute solution, is "freer" to do so on account of the lower osmotic pressure of the ions already in solution. For this reason it must become negatively charged to a greater degree, and the electric equilibrium having been upset, an e.m.f. is shown by the galvanometer.

Hitherto we have dealt with the production of e.m.f. when metals make contact with solutions of their salts; but an e.m.f. is similarly produced where certain gaseous elements make contact with aqueous solutions containing the same element in an ionised state. This may be demonstrated in the case of oxygen in contact with an alkaline solution, i.e., one containing dissociated OH, but for certain technical reasons a more general application has been found for a method in which hydrogen is brought into

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contact with solutions containing hydrogen ions, using this as a criterion of their comparative acidity.

In order to demonstrate this, advantage is taken of the property of condensing gases possessed by " platinum black," and the metal deposited in this form upon platinum or gold constitutes the electrode.

When such an electrode is placed in an atmosphere of hydrogen, the condensation of the gas on its surface is of such an order that the arrangement may be regarded as a solid rod of hydrogen, and, with certain technical restrictions, used as if it were such.

We may show the production of an e.m.f. with this electrode by setting up an arrangement similar to that described as a " concentration battery," only in this case means must be provided for keeping the platinum black saturated with hydrogen, while the two electrodes dip into solutions of an acid which differ in concentration from each other. A diagrammatic view of the apparatus is shown in Fig. 4.
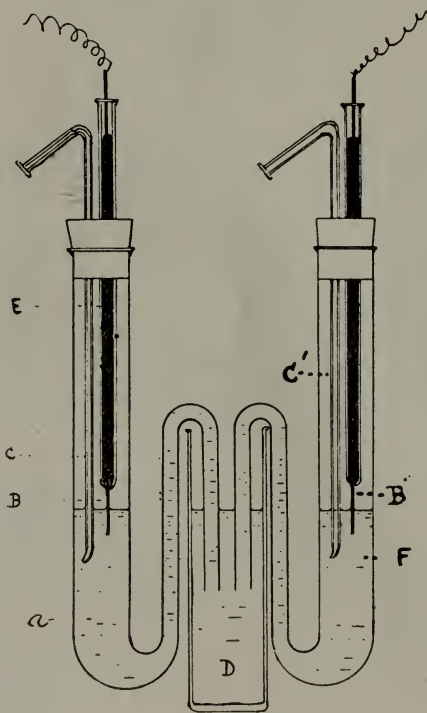

Fig. 4.

Let A be a vessel containing acid of known concentration, N/10 HCl for example, whose H ion concentration is known, while F represents a similar vessel containing acid of unknown strength. Dipping into the acid are two " hydrogen electrodes," B, B′, and means are provided whereby hydrogen is bubbled through the acid by means of the fine tubes CC′, creating an atmosphere of this gas about the exposed surfaces of the electrodes. The hydrogen is at atmospheric pressure, and serves to keep the electrodes in a state of saturation. Bent tubes from each electrode vessel make contact with a saturated solution of potassium chloride in the vessel D (the reason for which will be explained later), and wires from the electrodes complete a circuit which includes a delicate galvanometer.

On closing the circuit a current will flow in the direction of the vessel containing the weaker acid, since, in this case, the electrolytic solution pressure of hydrogen is greater on the electrode which is in contact with the acid of lesser concentration, i.e. of lesser hydrogen ion concentration.   Thus, the electrical balance is upset, this electrode having a lower potential than that in the vessel containing the stronger acid.   Such an arrangement is termed a " gas chain."

Nernst conceived the idea that these electrical phenomena might be treated quantitively, and he was able to construct a formula by which the hydrogen ion concentration of the unknown solution might be calculated, provided the e.m.f. developed, and the hydrogen ion concentration of the other solution were known.

In developing this formula Nernst was able to utilise the work of van't Hoff, who showed that if osmotic pressure be substituted for gas pressure in the formula $pv = RT$, the " gas laws " are applicable to substances in solution.   Their assimilation may be shown in the following way: Consider the case of a metal in contact with a solution of one of its salts, and call the " electrolytic solution pressure " of the metal P, and the osmotic pressure of the dissolved salt p; then the dissolved metal as it gives off ions may be regarded as going from pressure P to pressure p. Now, when a gram molecular weight of a gas expands isothermally from a pressure p_1 to another p_2, the amount of work done A, is expressed by the formula $A = RT \ln P_1/p_2$ where R is the gas constant, and T the absolute temperature.   Similarly, where a formula weight in grams of metal in dissolving and dissociating into ions, goes from solution pressure P to an osmotic pressure p, the maximum work is shown by the formula, $A = RT \ln P/_p$

If an equivalent weight of an univalent metal goes into solution, the charge carried by the ions will be 96540 coulombs, i.e., the Faraday constant F. However, total electricity is equal to quantity multiplied by intensity or potential; the formula will therefore be J P = RT $1n$ P/p or P = RT/F $1n$ P/p. Where the metal is not univalent, but has the valence n, this factor is introduced thus: P = RT/nF $1n$ P/p. (8). Now R, in electrical units, is 8.3 joules per degree, and F, in coulombs, 96540, while the absolute temperature T, is 291 (273 plus 18). The formula RT/F thus becomes:

$$\frac{8\cdot3 \times 291 \times 2\cdot3}{96540} = 0\cdot058v$$

The modulus 2.3 is introduced in order to bring natural to common logarithms. (9)

The significance of this equation is that a ten-fold change in the concentration would cause a difference of 0.058 v. in the electrode potential for a univalent ion, 0.029 for a bivalent, and 0.193 for a tervalent, and it certainly holds good for solution *below* decinormal strength. (10) The practical application of this will be considered later.

The e.m.f. developed by the hydrogen electrode varies within fairly narrow limits, and is never greater than a fraction over one volt. For the measurement of such currents where extreme accuracy is not called for, as in determining the end point of a reaction, or checking the normality of solutions used in volumetric analysis, a sensitive galvanometer may be used as described by Hildebrandt. (11)

In biochemical work, however, the standard practice is to employ the Poggendorf compensation method, using a potentiometer for this purpose.

In its simplest form (shown diagramatically in Fig. 5), the apparatus consists of a wire, *a, b,* of uniform calibre, stretched along a wooden " metre stick." To this wire a steady e.m.f. of about 1.5 volt is applied by means of an accumulator C. If now a cell D, whose e.m.f. it is desired to measure, be so connected to *ab,* that its e.m.f. is opposed to that of the cell C, and is supplied with a movable contact which slides along *ab,* then a point may be found where no deflection takes place in the galvanometer E, which is inserted in the circuit.

When this point is found, then the e.m.f. of the cell D is equal to the potential drop along AF, and it therefore corresponds to the ratio AF.FB.

In practice the potentiometer is constructed in such a way that a variable resistance is introduced between the accumulator C, and AB, the wire AB (or what corresponds to it) being placed against a scale which is graduated from zero to 1.5 volt. Instead of an unknown e.m.f. at D, a standard cell such as the Weston
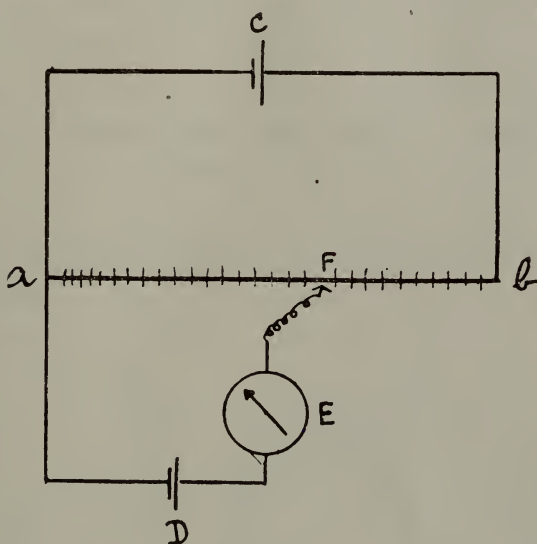

Fig. 5.

element is introduced, the movable contact F being placed on AB, at the known voltage of the cell used. When this is done, the variable resistance is adjusted until no deflection of the galvanometer E takes place. If now the source of current C be developing a steady e.m.f., the potential drop along the wire AB will correspond to the divisions on the scale, and the instrument will be ready for the determination of the unknown e.m.f. required.

To do this, the standard cell is cut out by means of suitable switches, and the source of the unknown e.m.f. (which may be a cell, or in this instance, the hydrogen electrode-calomel electrode used in these investigations) is thrown into the circuit. The sliding contact is now moved along AB until no deflection of the galvanometer occurs, and the required e.m.f. may be then read off on the scale.

In the concentration chain, and also in the gas chain previously referred to, an e.m.f. was shown to develop when electrodes were in contact with solutions which differed in the concentrations of the particular ion concerned, the two solutions being made part of an electric circuit.

In practice it would be possible to measure the H ion concentration of an unknown fluid, using for one side of the " chain " a solution of unit H ion concentration, i.e., corresponding to one gram ion per litre (12).

In the case of hydrogen, however, there is " much uncertainty in the reduction to normal ionic concentration," (10) and in routine work other means of completing the " cell " must be sought.

It will have become obvious that the hydrogen electrode which dips into the unknown fluid constitutes but one-half of the galvanic cell which must be constructed in order to determine the e.m.f. It is therefore necessary to complete the system by introducing as the other half, an electrode which develops a constant e.m.f. For this purpose the standard practice is to employ a " calomel electrode." For a full description of its preparation ⹂ ᵗext-book on the subject may be consulted.

It will be sufficient here to remark that these electrodes are named from the normality of the potassium chloride solutions used in them. The decinormal electrode has been very generally employed for this purpose, but the saturated calomel electrode has the advantage of easy preparation, and the absence of any need of a temperature correction. The latter has been almost exclusively employed by the writer, and has been found to meet all requirements. Its construction has followed the form suggested by Barendrecht. (13)

The materials used in making these electrodes have to be of extreme purity, and in connection with this, reference should be made to Findlay, (14) the classic article by Loomis and Acree (15), and the recent text book by W. M. Clark. (23)

As the e.m.f. of a calomel electrode varies with the concentration of the KCl solution employed, it is necessary to record on the electrode vessel the normality of the solution. For future reference it may be noted that the e.m.f. of a *hydrogen electrode* when the hydrogen ion concentration of the solution is normal, and when a saturated KCl electrode is employed, is 0.251 volt, whilst with the deci-normal KCl it is 0.3377 volt.

Some writers consider the potential of the calomel electrode as zero, but it is perhaps more convenient to call the normal hydrogen electrode zero, and then say that the potential of the calomel electrode is 0.251 volt, or whatever the figure may be.

A convenient form of calomel electrode suggested by Barendrecht is shown in fig. 6, where d is a glass jar, closed by a double bored rubber stopper, containing a saturated solution of potassium chloride. A test tube c perforated a short distance from the bottom contains a small quantity of pure mercury. Dipping below the surface of this a glass tube b provided with a sealed platinum wire enables one to effect electrical connection through a fine column of mercury contained in it. The glass tube e serves to connect the cell with a vessel containing saturated KCl. The stopcock g, provided it is not greased, may be kept closed during measurements. At f a plug of cotton wool prevents diffusion into c.
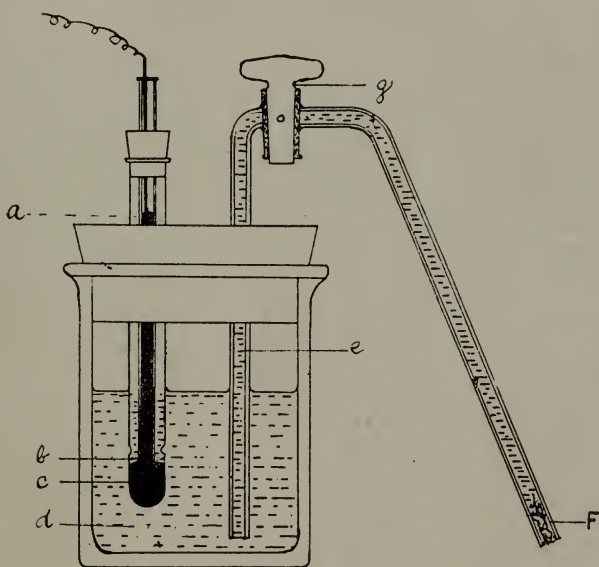

Fig. 6.

The connecting fluid.

For obvious reasons it is impossible to connect the calomel and hydrogen electrodes electrically by means of a metallic conductor. The usual practice is therefore to allow both electrodes to dip into some conducting solution. The proper selection of this

solution is a matter of great importance since serious errors would be introduced if reaction occurred between it and that in the electrodes. Some error is inevitable where solutions which are not identical are in contact, and in work which necessitates extreme accuracy, due allowance is made for this, but for routine biochemical estimations " contact potential " may be ignored. This has been made possible by the use of a saturated solution of KCl as a connecting fluid. In such a solution the speed of the dissociated ions is equal or nearly so; furthermore, where the saturated calomel electrode is employed, the solution is identical with that of the electrode, in which case error can occur only at the point where the fluid in the hydrogen electrode comes into contact with the connecting solution. For more detailed information the student is referred to the article by Michaelis. (7)

The Hydrogen Electrode.

The hydrogen electrode consists of a small element of pure platinum, or sometimes gold, which has had a layer of " platinum black " deposited upon it, and great care must be taken that the electrode is at all times completely platinised, and is not contaminated. The form given to the electrode varies according to the choice of the worker; thus it may be a small rectangular piece of foil, as used by Hildebrand (10) and Clark (16), or again it may be simply a fine platinum wire, the form preferred by Walpole (17) and Barendrecht (13). Under any circumstances, the electrode, when saturated with pure hydrogen, is placed in the solution in such a way that while part of it is immersed, a considerable portion is still exposed to the gas. Willsmore carried out his measurements with a rectangular piece of foil, which dipped for a considerable distance into the solution (19). It has been shown, however, that a steady e.m.f. is obtained more quickly when there is a minimum contact between the electrode and the solution. The reason for this is not at once apparent, but it is supposed to depend on certain peculiar physical conditions at the surface of the liquid, whereby a rapid diffusion from the surrounding solution to the point of contact is prevented, the local conditions maintaining an equilibrium, which is not quickly upset (16). As minimum contact is easily obtained with the electrode devised by Walpole, this electrode, modified by Barendrecht, has been most

generally used in this laboratory. It has been shown by Has-
selbalch that a steady e.m.f. is rapidly obtained when the elec-
trode vessel is shaken, thus bringing all the solution into contact
with the electrode, and he has devised special apparatus for
doing this. (20)   The Hasselbalch electrode has undergone
some modification at the hands of other workers, and of these,
that constructed by Clark has found general acceptance. A full
description of the electrode will be found in the article by
Clark. (16)

Hydrogen Electrode.

The Barendrecht electrode consists of a capillary glass tube,
having an elongated bulb of about 0.5 c.c. capacity blown upon
it. A platinum wire is sealed in through the side of the bulb,
and bending at right angles, approaches the construction in the
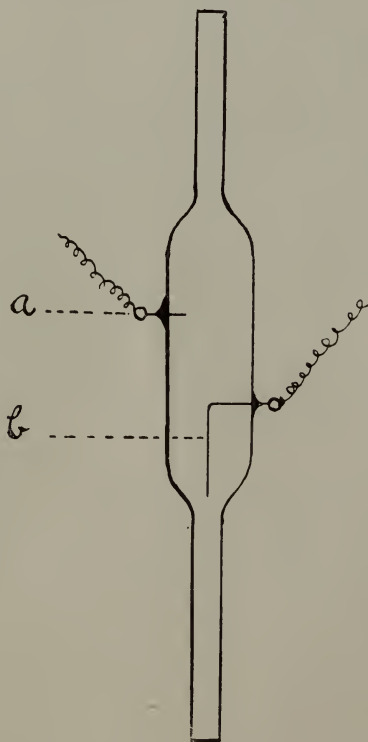lower part. This is shown in Fig. 7.

FIG. 7.

The platinum wire at B has a fine copper wire soldered to it, which is led away to a potentiometer. In experimenting with the electrode, the writer was led to make several modifications in its construction. In the first place, it was found that in platinising the electrode, the resistance of the solution in the fine part of the tube was a disadvantage. To obviate this, the writer sealed what may be termed an " inert electrode " into the tube at A. This is used only during platinising, constituting the anode in the system, and the arrangement, when used with a platinising vessel, to be described later, makes for convenience and ease of operation.

Another modified electrode is shown in Figure 8, which, in this case is provided with two hydrogen electrodes, and one inert electrode. It is thus possible to compare the e.m.f.'s of two electrodes simultaneously placed in the solution under examination, and with it one learns how easily an incorrect reading may be given by an electrode which, to all appearances, is properly platinised, and is in good working order.
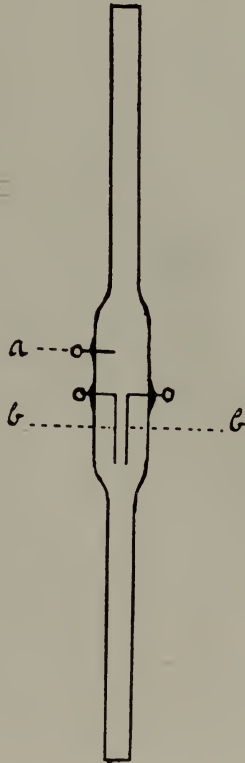

Fig. 8.

The Platinizing of the Electrodes.

The platinum electrodes for hydrogen ion estimation which have just been described, are coated with platinum black in the following manner.

First, the platinum is thoroughly cleared from grease and other foreign matter by immersion in a potassium bichromate-sulphuric acid mixture. It is then washed and connected to the negative pole of an accumulator or suitable source of current. If the electrode is of the removable type, such as the Walpole Hasselbalch, or the Clark, it is now placed in a beaker, and covered with the platinising solution, the current from the positive side being led into the solution by a clean platinum wire. The platinising solution must be prepared with due precautions as to its chemical purity, and consists of a 3 per cent. solution of platinic chloride, with a trace of lead acetate added to it. A new electrode will require to be immersed possibly for five minutes, and some operators suggest the coating should be so thick that part will fall off. When the electrode is completely coated with platinum black, it must be well washed, after which it is again connected to the cell, and made the negative electrode in a beaker containing dilute sulphuric acid, a platinum wire as before, constitutes the anode. The current should be sufficient to cause vigorous bubbling. By this means all traces of platinum chloride will be removed from the coating on the electrode.(7) It must now be well washed in a stream of distilled, or preferably " conductivity " water, and afterwards kept in a beaker of the same from contamination. Prolonged washing of the electrode is not necessary after this treatment. The writer has devised an arrangement which makes the platinizing of the Barendrecht or modified Barendrecht type of electrode comparatively simple.

It is illustrated in figure (9) where *e* is a vessel closed by a double bored cork, *a* is the electrode tube, while to *d* a rubber tube is connected, which is kept closed with a clamp. In using the apparatus, one blows through *d*, and forces the level of the platinising solution above the electrodes in *a*. As the electrode tube is thus kept vertical during platinisation, the bubbles of gas coming away from the electrodes are free to escape at the upper end of the tube. With other methods of coating, this bubbling about the electrodes was a great source of trouble. An exactly similar glass vessel is provided, to which the electrode is trans--

ferred, subsequent to its platinising, and after a thorough washing in distilled water.

The second vessel is provided with a 5 per cent. solution of sulphuric acid, and when the electrodes have been connected
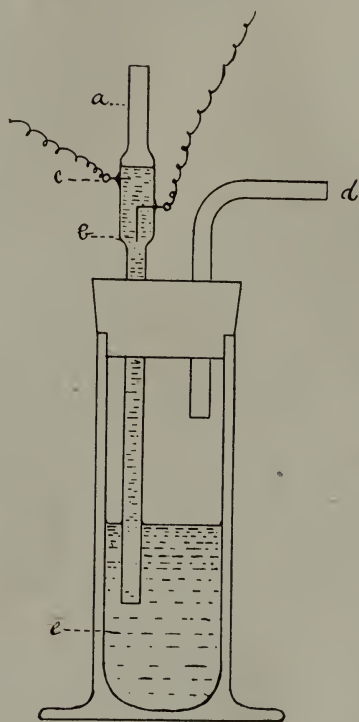

Fig. 9.

as before, i.e., making the hydrogen electrode negative in the arrangement, it is left to bubble vigorously for about ten minutes. It must now receive a most careful washing, and after saturation with hydrogen, be preserved in a beaker of distilled water.

As Michaelis points out, the hydrogen electrode does not need replatinising frequently when protein solutions only are used.

It does not readily make small errors, but if out of order will give quite impossible results. A deterioration of the electrode is indicated when it takes a long time to obtain a constant reading. In taking readings Michaelis recommends that they should be made every ten minutes, and should not be accepted until three successive readings are identical. Before

use in routine investigations, the hydrogen electrode must be subjected to a test in order to ascertain if it be in good working order. To do this one must place in the electrode vessel a solution of known hydrogen ion concentration, and for this purpose Michaelis suggests a "buffer" mixture made as follows:—

>   10 c.c. N   Na OH.
>   20 c.c. N   HA.
>   70 c.c. distilled water.

Using the N/10 KCL calomel electrode this mixture should, at 18°C. give an e.m.f. of 0.6045 v. At other temperatures the e.m.f. of this chain is as follows:—

>   20 c. .. ..   0.6061 v.
>   22 c. .. ..   0.6078 v.
>   24 c. .. ..   0.6093 v.

That is to say, there is a rise of 0.8 millivolt for every degree of temperature. (7)

Examination of Fluids Containing Gases in Solution.

The presence of such gases as oxygen, chlorine, ammonia, carbon dioxide or nitrogen in solutions makes their examination by the electrometric method difficult, or in certain cases, impossible.

Chlorine gas in such a fluid for instance, would partly saturate the electrode, and also diffuse into the gas space above. In this case, the e.m.f. would be in part due to its presence, thus making the result worthless from the point of view of hydrogen ion concentration estimation.

Ammonia is a gas, the presence of which makes an examination by this method impossible.

The occurrence of CO_2 in solution is common enough in physiological fluids, and it is of some importance that the effect of its presence on the e.m.f. developed be noted. The diffusion of CO_2 into the gas space above the fluid will lower the partial pressure of the hydrogen, and as the electrolytic solution pressure of hydrogen is a function of its partial pressure, the reading on the potentiometer would not be an accurate measure of the hydrogen ion concentration of the solution. As to whether the error thus introduced is sufficient to be of importance, authorities differ. Milroy, in carrying out some important investi-

gations in the changes of hydrogen ion concentration in the blood, was content to disregard it. He remarks that the error must be small, since the measured e.m.f. varies only as the logarithm of the partial pressure, and in his opinion its recognition did not warrant the needless bleeding of the animal involved in the method of Hasselbalch (5).

Where it is thought absolutely necessary to eliminate this source of error, recourse may be had to the Konikoff or Hasselbalch electrode vessel.

These vessels provide means whereby the specimen being examined may be brought into contact with the hydrogen atmosphere above the electrode and shaken, thus allowing the tension of the CO_2 in the hydrogen to become equal to that in the fluid. After this has been done, the fluid is removed, and replaced by a fresh sample without changing the hydrogen atmosphere. There will then be no further diffusion of CO_2 into the gas space, since conditions of equal tension will have been established as regards any CO_2 present. Incidentally, the shaking of the electrode tube will have the effect of removing any oxygen present in the liquid, by combining it with the hydrogen of the electrode. This in itself will assist in obtaining a constant e.m.f. in a comparatively short time.

Preparation of the Hydrogen.

The hydrogen used in these tests must be of extreme purity. If it be prepared from dilute sulphuric acid and zinc in Kipp's apparatus, there may be some danger of contamination with hydrogen sulphide. Michaelis suggests that the gas be washed with potassium permanganate solution, to remove any oxidisable substance, and then bubbled through a solution of mercuric chloride. (7)

Sharp and Hoagland prepare the hydrogen by electrolysing a 25 per cent by weight solution of caustic potash in a special cell, using nickel electrodes. The hydrogen is led away, and passed through a tube containing platinum black deposited upon asbestos, which is kept hot by means of a glowing spiral of high resistance wire electrically heated, the purpose of which is to remove, as water, any oxygen which might be present. (6)

In this laboratory, hydrogen, electrolytically prepared, and stored in steel cylinders, is exclusively employed for saturating the electrodes.

The Operation of the Electrode.

As has previously been remarked, in this laboratory, the simple electrode devised by Barendrecht has been used to the exclusion of all others in routine work. In Barendrecht's original article it will be seen that the fluid is drawn up and down the glass vessel by a close fitting syringe. This is shown in Fig. 10. To
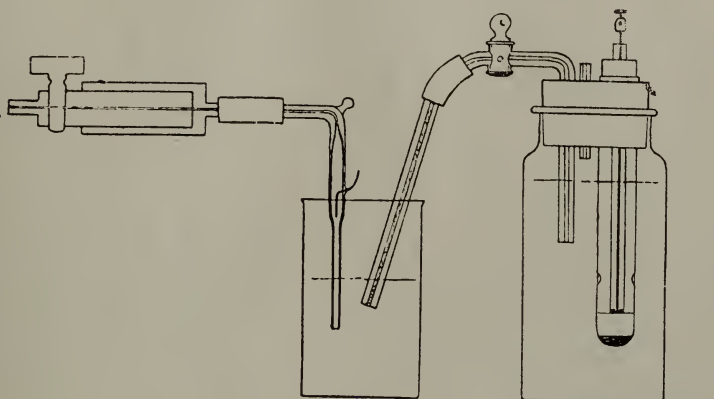

FIG. 10.   After Barendrecht

do this by hand in the usual way made it difficult at times to affect the fine adjustment needed to obtain a minimum contact. The writer has therefore devised a ratchet and pinion attachment, which is connected to the plunger of the syringe, which allows a very exact adjustment to be made with but little trouble.

A diagramatic view of the device is shown in fig. 11, where a is the " syringe," and b the perforated plunger, which is controlled through the rackwork by means of the milled wheel g. The hydrogen is admitted at c, and the glass electrode tube c is attached closely to the apparatus by a rubber tube d. The whole arrangement is made adjustable on the stand f.

The technique of using this apparatus is as follows:—Having thoroughly ventilated the electrode with hydrogen, and before the fluid to be investigated has been drawn up the tube, the plunger should be moved in and out by means of the milled wheel in order to remove any trace of air which might be occluded between it and the end of the tube; then, with the hydrogen passing through the electrode slowly, the open end of the glass tube is brought below the surface of the liquid to be
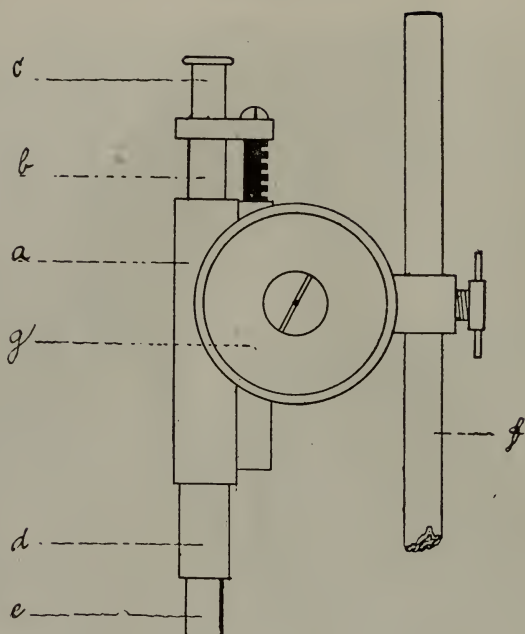
Fig. 11.

examined. Immediately this is done, the gas should be ·cut off,. the cock on the piston being provided for this purpose. The piston is then gently drawn out of the barrel, and the solution · allowed to enter the vessel, and completely to cover the platinum wire. Keeping the end of the vessel still below the surface of the liquid, the contained fluid is ejected from the tube, allowing a minute portion to remain in the capillary end. Another portion is now drawn up and down the platinum wire, and again ejected, after which the final portion is allowed to enter the vessel in such a manner that the platinum wire makes a minimum contact with the fluid. In dealing with liquids which do not contain carbon dioxide, it is not perhaps so necessary to leave a small quantity in the tube as described, since the object of this is to retain the original hydrogen atmosphere, plus carbon dioxide in equilibrium with that contained in the fluid. The reason for " washing " the platinised wire with the fluid of the final portion is, that in this way the contained oxygen is reduced by the hydrogen of the wire, and a steady e.m.f. is sooner obtained, also the changing of the solution should enable the CO_2 in the final portion to be in equilibrium with that in the hydrogen, and thus do way with any risk of diffusion from

fluid into gas space. The electrode tube is now wiped with filter paper in order to remove any fluid adhering to it, and the vessel containing the connection solution is brought below it, the tube making minimal contact with the solution. The tube connected with the calomel electrode having also been brought below the surface of the connecting fluid, the operator may now proceed to measure the resultant e.m.f. according to the directions supplied with the particular potentiometer used.

In recording these measurements, a note should be made of the time elapsing between each test, a convenient interval being five minutes. In general it will be noticed that during the first few minutes there will be a gradual increase in the e.m.f. This effect is less noticeable with those electrodes which employ a shaking device, and if such be used, it would appear to be desirable to depend upon measurements taken a short time after the preliminary shaking rather than upon the record of a single long experiment. This matter of " potential drift " is treated at length in the article by Clark, previously referred to.

Between each experiment the electrode tube must be thoroughly washed with distilled water; it should be then saturated with hydrogen, and immersed in a beaker of distilled water in which it is kept until required.

The Calculation of the Result.

Having obtained the e.m.f. on the potentiometer scale, the H. ion concentration is calculated from it by means of Nernst's formula as follows, to quote Milroy:—

Let π_p = the e.m.f. of the system.

π_0 = the e.m.f. of the calomel electrode employed when the H$^{\cdot}$ concentration is normal, viz., 0.251 volt at 18 C., using the saturated KCl calomel electrode; while C, and C_0 are the H$^{\cdot}$ ion concentrations of the two fluids.

Then—
$$\pi_p = \pi_0 + .0577.\log.\frac{Co.}{C_{p.}}$$

C may therefore be calculated when π is measured.

Or, if P be substituted for Cp (10^{-p}), the result may be made to serve as a basis of measurement.

So, then—
$$\frac{\pi_p - .251}{.0577} = \log. \frac{1}{C} \text{ or } -\log. C.$$

The value $^{-}\log$ C is, in accordance with the suggestion of Sörensen, commonly employed to express the H$^{\cdot}$ ion concentra-

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tion, and has received the name " Sörenson's negative hydrogen ion exponent," and is indicated by the sign P_H, or pH.

In dealing with such a notation, one must bear in mind the fact that a rise in the P_H signifies a fall in the $H\cdot$ ion concentration. (5) As Baylis remarks, while it is " easy to see that a hydrogen ion concentration of $4x10^{-6}$ is double that of $2x10^{-6}$, it is not at once obvious that a P_H of 5.398 is double that of 5.699. One has to get accustomed to thinking in negative logarithms." (1)

To this end it may perhaps be helpful to the student if the pH of familiar laboratory reagents such as decinormal hydrochloric and acetic acids is calculated.

We may do this if we know the normality of the solution, and its degree of dissociation.

As Macleod points out, decinormal hydrochloric acid is dissociated 91 per cent., it is therefore 0.091 N or $9.1x10^{-2}$. The P_H is found by subtracting from the power of ten, the logarithm of the reciprocal of the normality in hydrogen ions. Thus:— Log. 9.1 is .96, now -2 minus $.96 = -1.04$, which is the pH sought.

Again, acetic acid in decinormal solution is dissociated 1.3 per cent; it is therefore 0.0013 N in hydrogen ions, or $1.3x10^{-3}$. Now, log. 1.3 is .11 ; subtracting .11 from -3, we get -2.89, and the pH is therefore 2.89.*

To find the normality in ordinary notation, we must subtract the pH from the next higher whole number, and take the antilogarithm of this number.

Thus, if the P_H be 7.45, subtracting this number from 8, we get 0.55. Now, the antilogarithm of this is 3.55, and the hydrogen ion concentration, or C_H is therefore $3.55x10^{-8}$. (21)

We would again lay stress on the fact previously mentioned, i c., the formula of Nernst shows that for a tenfold difference in the hydrogen ion concentration of the fluid under examination, the electrode potential changes by only $0.058\cdot$ volt.

In the case of the concentration cell previously mentioned, it is found that " when a univalent electrolyte is employed. an e.m.f. of 0.058 volt is developed where one solution is ten times the concentration of the other, and the electrode potential between monad metal and a normal solution of the corresponding metallion is thus 0.058 volt smaller than the electrode potential between the same metal, and a decinormal solution of the metallion." (4)

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* Algebraic sum would probably be the better expression to use in these cases.

Schmidt (22) has discussed the matter of *p*H in a way which makes the use of this notation as a measure of reaction, more intelligible to the student. He says, " the term *p*H is given to the exponent of ten taken as a positive number. This is the most rational system, since all values are expressed in the same units. Thus $C_H = 5.03 \times 10^{-10}$ can be expressed entirely as a power of ten.

$$5 \cdot 03 = 10^{0 \cdot 702}(\text{ since } \log._{10} 5 \cdot 03 = 0 \cdot 702)$$
$$C_H = 10^{0.702} \times 10^{-10}$$
$$= 10^{-9.298}$$

Therefore $P_H = 9 \cdot 298.$

Another example : To find P_H when $C_H = 0 \cdot 409 \times 10^{-7}$—

$$P_H = \log._{10} \frac{1}{C_H}$$
$$P_H = \log._{10} 1 - \log._{10} CH$$
$$\log._{10} C_H = 10^{-7} \times 10^{1 \cdot 612}$$
$$= \overline{8} \cdot 612$$
$$P_H = 17 \cdot 388 - 10$$
$$P_H = 7 \cdot 388.$$

Or, starting from the log. $\overline{8}.612$, one may convert this into the negative form by subtracting from 0.000 and obtain the required result, thus :—

$$0.000$$
$$\overline{8}.612$$
$$\overline{}$$
$$-7.388$$

Which is the required *p*H.

A useful method of calculating the hydrogen concentration decimal points of the *p*H from right to left as abscissae, while it one draws a diagonal line on semi-log paper, and plots the first decimal points of the *p*H from right to left as abscissae, while the hydrogen ion concentrations are plotted, from 0.1 to 1.0 as ordinates.

If, for example, one wishes to convert *p*H 6.7 into a corresponding hydrogen ion concentration, one finds by examination that 6.7 cuts the diagonal line at 0.2, and the concentration is 0.2×10^{-6}.

By plotting the electrical potentials against the hydrogen ion concentration on semi-log paper one can, if necessary, avoid the use of S¹rensen's exponent or *p*H.

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In conclusion the writer would like to put on record his appreciation of the many kindnesses extended to him over a number of years by Prof. Osborne, but for whose encouragement and help this work could not have been undertaken.

The author has not hesitated to quote freely from the authorities named, and trusts that the student may be led to read at length the articles referred to.

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